

## **REDEPOSITION OR BACKSTAIN INHIBITION DURING STONEWASHING PROCESS**

### **CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a continuation application of PCT/DK01/00384 filed June 5, 2001 (the international application was published under PCT Article 21(2) in English) and claims, under 35 U.S.C. 119, priority or the benefit of Danish application nos. PA 2000 00861, PA 2000 01577, PA 2000 01772 and PA 2001 00100 filed June 2, 2000, October 23, 2000, November 24, 2000 and January 19, 2001, respectively, and U.S. provisional application nos. 60/211,004, 60/244,351, 60/253,798, and 60/265,473 filed on June 12, 2000, October 30, 2000, November 29, 2000, and January 31, 2001 respectively, the contents of which are fully incorporated herein by reference.

### **FIELD OF INVENTION**

The present invention relates to compositions and methods for reducing or preventing the backstaining of dye on textile materials, especially indigo on denim and specially the backstaining of pocket parts of denim during the stonewashing of denim fabric.

### **BACKGROUND OF THE INVENTION**

By stonewashing of denim the usually blue-dyed denim is given a faded or worn appearance with the characteristic white and blue contrast. Stonewashing the denim material is typically carried out in the presence of pumice stone or cellulase or a combination thereof and results in the removal of dye to give areas of lighter color. The use of cellulase instead of pumice stone has the advantages that it is more environmental friendly, more economical and prevents that the denim is damage because of the rough treatment with the pumice stones. However, the use of cellulase is not without disadvantages.

The dye removed from the denim material after the treatment with cellulase or by a conventional washing process may cause "backstaining" or "redeposition" onto the denim material, e.g. re-coloration of the blue threads and blue coloration of the white threads, resulting in a less contrast between the blue and white threads. In order to remove the dye the denim manufactures are using huge amount of surfactants to make parts white again at a soaping process with heavy washing condition. The heavy washing condition causes color change or color-fading problems for finished denim products. Also additional water has to be used in the subsequent soaping process.

The problem of redeposition or backstaining of dye during stonewashing has also been addressed by adding anti-redeposition chemicals, such as surfactants or other agents into the cellulase wash. Also the use of different cellulases with less specific activity on denim has

been tried. WO-A-9407983 describes the use of a cellulase to inhibit the backstaining of denim. WO-A-9429426 and WO-A-9325655 describes backstain inhibition by treatment with a redeposition cellulase composition and added protease as an improvement over the use of redeposition cellulase alone.

Although, these methods aim to solve the problem with the backstaining or redeposition of dye onto the denim material, they may still be improved. In particular, the backstaining or redeposition of dye onto the pocket parts of the denim material poses a problem.

## **SUMMARY OF THE INVENTION**

We have developed a process for treating fabric, especially indigo-dyed denim, with a composition comprising a lipolytic enzyme.

This treatment reduces the risk of back-staining (redeposition of dye onto textile) even when less water is used. The enzymatic treatment of released dyestuff will decrease process time as well as the amount of energy and water needed to achieve a satisfactory quality of the textile, and the color of the wastewater is reduced.

The method of the invention can result in a decreased number of washes, thereby increasing the productivity and decreasing the consumption of water and chemicals, including surfactants.

Accordingly, the present invention provides a method for reducing the backstaining of fabric or textile, comprising contacting the fabric or textile with a composition comprising an effective amount of a lipolytic enzyme (EC 3.1.1).

In another aspect, the present invention relates to a stonewashing composition comprising a lipolytic enzyme and a cellulase.

In a third aspect, the invention relates to the use of the composition for reducing backstaining of fabric or textile.

## **DETAILED DISCLOSURE OF THE INVENTION**

Denim that is stonewashed with the addition of an effective amount of added lipolytic enzyme during cellulase treatment shows a reduction in the level of backstaining, especially the backstaining of pocket parts.

The method of the present invention comprises contacting the denim to be enzymatically stonewashed with a composition comprising the lipolytic enzyme in a amount sufficient to reduce backstaining and thus, to decrease the blue-coloring of e.g. the pocket parts.

The amount of added lipolytic enzyme depends upon others on the purity and amount of cellulase used in the stonewashing process, the contact time, the amount of dye removed during stonewashing, the activity of the cellulase, the pH and temperature of the stonewashing process, the formulation of the product and the like.

The composition to be added may further comprise various adjuvants as known to the skilled person, e.g. surfactants. Other materials can also be used with the composition as desired, including stones, fillers, solvents, buffers, pH control agents, enzyme activators, builders, enzyme stabilizers, other anti-deposition agent and the like. The composition may be formulated at a solid product, granular product or as a liquid product.

The lipolytic enzyme may be added to the composition containing the cellulase for use in stonewashing process or added directly to the stonewashing bath or to a subsequent rinse treatment. The lipolytic enzyme may also be added to a composition for washing purposes thereby reducing or inhibiting the backstaining of removed dye during the washing process.

#### Fabrics

The process of the present invention applies to fabrics in general. In the context of this invention fabrics include fabrics or textiles prepared from man-made fibers, e.g. polyester, nylon, etc., as well as cellulosic fabrics or textiles.

The term "cellulosic fabric/textile" indicates any type of fabric, in particular woven fabric, prepared from a cellulose-containing material, containing cellulose or cellulose derivatives, e.g. from wood pulp, and cotton. The main part of the cellulose or cellulose derivatives present on the fabric is normally size with which the yarns, normally warp yarns, have been coated prior to weaving. In the present context, the term "fabric" is also intended to include garments and other types of processed fabrics. Examples of cellulosic fabric is cotton, viscose (rayon); lyocell; all blends of viscose, cotton or lyocell with other fibers such as polyester; viscose/cotton blends, lyocell/cotton blends, viscose/wool blends, lyocell/wool blends, cotton/wool blends; flax (linen), ramie and other fabrics based on cellulose fibers, including all blends of cellulosic fibers with other fibers such as wool, polyamide, acrylic and polyester fibers, e.g. viscose/cotton/polyester blends, wool/cotton/polyester blends, flax/cotton blends etc. The fabric may also include man-made fibers alone such as polyester fibers.

The process of the invention is preferably applied to cellulose-containing fabrics, such as cotton, viscose, rayon, ramie, linen or mixtures thereof, or mixtures of any of these fibers with synthetic fibers. In particular, the fabric may be denim. The fabric may be dyed with vat dyes such as indigo, direct dyes such as Direct Red 185, sulfur dyes such as Sulfur Green 6, or reactive dyes fixed to a binder on the fabric surface. In a preferred embodiment

of the present process, the fabric is indigo-dyed denim, including clothing items manufactured therefrom.

In a most preferred embodiment, the fabric subjected to the process of the invention is made of hydrophobic fibres such as polyamide fibres, e.g. nylon, acrylic fibres, vinylon and polyester fibres. As mention above the fabric may be made of mixtures of different fibres. Especially contemplated is polyester or polyester/cotton mixtures, which are the material used for pocket parts of garments, in particular dyed cotton garments or denim jeans.

### Enzyme

The enzymatic process of the invention may be accomplished using any carboxylic ester hydrolases, in particular lipolytic enzyme and/or any biopolyester hydrolytic enzyme. Such enzymes are well known and defined in the literature, cf. e.g. *Borgström B and Brockman H L*, (Eds.); Lipases; Elsevier Science Publishers B.V., 1984, and *Kolattukudy P E*; The Biochemistry of Plants, Academic Press Inc., 1980, 4 624-631.

In the context of this invention lipolytic enzymes are classified in E.C. 3.1.1 and include true lipases, esterases, phospholipases, and lyso-phospholipases. More specifically the lipolytic enzyme may be a lipase as classified by EC 3.1.1.3, EC 3.1.1.23 and/or EC 3.1.1.26, an esterase as classified by EC 3.1.1.1, EC 3.1.1.2, EC 3.1.1.6, EC 3.1.1.7, and/or EC 3.1.1.8, a phospholipase as classified by EC 3.1.1.4 and/or EC 3.1.1.32, a lyso-phospholipase as classified by EC 3.1.1.5 and a cutinase as classified in EC 3.1.1.74.

The lipolytic enzyme preferably is of microbial origin, in particular of bacterial, of fungal or of yeast origin.

In a particular embodiment, the lipolytic enzyme used may be derived from a strain of *Absidia*, in particular *Absidia blakesleena* and *Absidia corymbifera*, a strain of *Achromobacter*, in particular *Achromobacter iophagus*, a strain of *Aeromonas*, a strain of *Alternaria*, in particular *Alternaria brassiciola*, a strain of *Aspergillus*, in particular *Aspergillus niger* and *Aspergillus flavus*, a strain of *Achromobacter*, in particular *Achromobacter iophagus*, a strain of *Aureobasidium*, in particular *Aureobasidium pullulans*, a strain of *Bacillus*, in particular *Bacillus pumilus*, *Bacillus stearothermophilus* and *Bacillus subtilis*, a strain of *Beauveria*, a strain of *Brochothrix*, in particular *Brochothrix thermosohata*, a strain of *Candida*, in particular *Candida cylindracea* (*Candida rugosa*), *Candida paralipolytica*, *Candida tsukubaensis*, *Candida auriculariae*, *Candida humicola*, *Cadida foliarum*, *Candida cylindracea* (*Cadida rugosa*) and *Candida antarctica*, a strain of *Chromobacter*, in particular *Chromobacter viscosum*, a strain of *Coprinus*, in particular *Coprinus cinerius*, a strain of *Fusarium*, in particular *Fusarium oxysporum*, *Fusarium solani*, *Fusarium solani pisi*, and *Fusarium roseum culmorum*, a strain of

*Geotricum*, in particular *Geotricum penicillatum*, a strain of *Hansenula*, in particular *Hansenula anomala*, a strain of *Humicola*, in particular *Humicola brevispora*, *Humicola lanuginosa*, *Humicola brevis* var. *thermoidea*, and *Humicola insolens*, a strain of *Hyphozyma*, a strain of *Lactobacillus*, in particular *Lactobacillus curvatus*, a strain of *Metarhizium*, a strain of *Mucor*, a strain of *Paecilomyces*, a strain of *Penicillium*, in particular *Penicillium cyclopium*, *Penicillium crustosum* and *Penicillium expansum*, a strain of *Pseudomonas* in particular *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, *Pseudomonas cepacia* (syn. *Burkholderia cepacia*), *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Pseudomonas maltophilia*, *Pseudomonas mendocina*, *Pseudomonas mephitica lipolytica*, *Pseudomonas alcaligenes*, *Pseudomonas plantari*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas putida*, *Pseudomonas stutzeri*, and *Pseudomonas wisconsinensis*, a strain of *Rhizoctonia*, in particular *Rhizoctonia solani*, a strain of *Rhizomucor*, in particular *Rhizomucor miehei*, a strain of *Rhizopus*, in particular *Rhizopus japonicus*, *Rhizopus microsporus* and *Rhizopus nodosus*, a strain of *Rhodospiridium*, in particular *Rhodospiridium toruloides*, a strain of *Rhodotorula*, in particular *Rhodotorula glutinis*, a strain of *Sporobolomyces*, in particular *Sporobolomyces shibatanus*, a strain of *Thermomyces*, in particular *Thermomyces lanuginosus* (formerly *Humicola lanuginosa*), a strain of *Thiarosporella*, in particular *Thiarosporella phaseolina*, a strain of *Trichoderma*, in particular *Trichoderma harzianum*, and *Trichoderma reesei*, and/or a strain of *Verticillium*.

In a more preferred embodiment, the lipolytic enzyme used according to the invention is derived from a strain of *Aspergillus*, a strain of *Achromobacter*, a strain of *Bacillus*, a strain of *Candida*, a strain of *Chromobacter*, a strain of *Fusarium*, a strain of *Humicola*, a strain of *Hyphozyma*, a strain of *Pseudomonas*, a strain of *Rhizomucor*, a strain of *Rhizopus*, or a strain of *Thermomyces*.

In a more preferred embodiment, the lipolytic enzyme used according to the invention is derived from a strain of *Bacillus pumilus*, a strain of *Bacillus stearothermophilus* a strain of *Candida cylindracea*, a strain of *Candida antarctica*, in particular *Candida antarctica* Lipase B (obtained as described in WO 88/02775), a strain of *Humicola insolens*, a strain of *Hyphozyma*, a strain of *Pseudomonas cepacia*, or a strain of *Thermomyces lanuginosus*.

In the context of this invention biopolyester hydrolytic enzyme include esterases and poly-hydroxyalkanoate depolymerases, in particular poly-3-hydroxyalkanoate depolymerases. In fact an esterase is a lipolytic enzyme as well as a biopolyester hydrolytic enzyme.

In a more preferred embodiment, the esterase is a cutinase or a suberinase. Also in the context of this invention, a cutinase is an enzyme capable of degrading cutin, cf. e.g. *Lin T S & Kolattukudy P E, J. Bacteriol.* 1978, 133 (2) 942-951, a suberinase is an enzyme capable of degrading suberin, cf. e.g. , *Kolattukudy P E; Science* 1980, 208 990-1000, *Lin T S &*

*Kolattukudy P E*; Physiol. Plant Pathol. 1980, 17 1-15, and The Biochemistry of Plants, Academic Press, 1980, Vol. 4 624-634, and a poly-3-hydroxyalkanoate depolymerase is an enzyme capable of degrading poly-3-hydroxyalkanoate, cf. e.g. *Foster et al.*, FEMS Microbiol. Lett. 1994, 118 279-282. Cutinases, for instance, differs from classical lipases in that no measurable activation around the critical micelle concentration (CMC) of the tributyrine substrate is observed. Also, cutinases are considered belonging to a class of serine esterases.

The biopolyester hydrolytic enzyme preferably is of microbial origin, in particular of bacterial, of fungal or of yeast origin.

In a preferred embodiment, the biopolyester hydrolytic enzyme is derived from a strain of *Aspergillus*, in particular *Aspergillus oryzae*, a strain of *Alternaria*, in particular *Alternaria brassiciola*, a strain of *Fusarium*, in particular *Fusarium solani*, *Fusarium solani pisi*, *Fusarium roseum culmorum*, or *Fusarium roseum sambucium*, a strain of *Helminthosporum*, in particular *Helminthosporum sativum*, a strain of *Humicola*, in particular *Humicola insolens*, a strain of *Pseudomonas*, in particular *Pseudomonas mendocina*, or *Pseudomonas putida*, a strain of *Rhizoctonia*, in particular *Rhizoctonia solani*, a strain of *Streptomyces*, in particular *Streptomyces scabies*, or a strain of *Ulocladium*, in particular *Ulocladium consortiale*. In a most preferred embodiment the biopolyester hydrolytic enzyme is a cutinase derived from a strain of *Humicola insolens*, in particular the strain *Humicola insolens* DSM 1800.

In another preferred embodiment, the poly-3-hydroxyalkanoate depolymerase is derived from a strain of *Alcaligenes*, in particular *Alcaligenes faecalis*, a strain of *Bacillus*, in particular *Bacillus megaterium*, a strain of *Camomonas*, in particular *Camomonas testosteroni*, a strain of *Penicillium*, in particular *Penicillium funiculosum*, a strain of *Pseudomonas*, in particular *Pseudomonas fluorescens*, *Pseudomonas lemoignei* and *Pseudomonas oleovorans*, or a strain of *Rhodospirillum*, in particular *Thodospirillum rubrum*.

Specific examples of readily available commercial lipases include Lipolase® (WO 98/35026) Lipolase™ Ultra, Lipozyme®, Palatase®, Novozym® 435, Lecitase® (all available from Novozymes A/S).

Examples of other lipases are Lumafast™, *Ps. mendocian* lipase from Genencor Int. Inc.; Lipomax™, *Ps. pseudoalcaligenes* lipase from Gist Brocades/Genencor Int. Inc.; *Fusarium solani* lipase (cutinase) from Unilever; *Bacillus sp.* lipase from Solvay Enzymes. Other lipases are available from other companies.

## Process conditions

In the case of denim textiles (especially indigo-dyed denim), the process according to the invention can be carried out simultaneously with a treatment with cellulase (and optionally pumice) to create a desired worn look by forming local variations in color density, as described

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### Cellulytic Activity

The cellulytic activity may be measured in endoglucanase units (EGU), determined at pH 6.0 with carboxymethyl cellulose (CMC) as substrate.

A substrate solution is prepared, containing 34.0 g/l CMC (Hercules 7 LFD) in 0.1 M phosphate buffer at pH 6.0. The enzyme sample to be analyzed is dissolved in the same buffer. 5 ml substrate solution and 0.15 ml enzyme solution are mixed and transferred to a vibration viscosimeter (e.g. MIVI 3000 from Sofraser, France), thermostated at 40°C for 30 minutes.

One EGU is defined as the amount of enzyme that reduces the viscosity to one half under these conditions. The amount of enzyme sample should be adjusted to provide 0.01-0.02 EGU/ml in the reaction mixture. The arch standard is defined as 880 EGU/g.

The cellulytic activity may also be determined in endo-cellulase units (ECU) by measuring the ability of the enzyme to reduce the viscosity of a solution of carboxymethyl cellulose (CMC).

The ECU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxymethylcellulose (CMC). The assay is carried out at 40°C; pH 7.5; 0.1 M phosphate buffer; time 30 min; using a relative enzyme standard for reducing the viscosity of the CMC Hercules 7 LFD substrate; enzyme concentration approx. 0.15 ECU/ml. The arch standard is defined to 8200 ECU/g.

### Color Measurement

A Nippon Denshoku's spectrophotometer (SE 2000), which was in accordance with JIS Z8722, ASTM E308, ASTM E313 and ASTM D1925, was used according to the manufacturer's instructions to evaluate the chromaticity using the change in the color space coordinates  $L^*a^*b^*$  (CIELAB-system), where as usual:

$L^*$  gives the change in white/black on a scale from 0 to 100, and a decrease in  $L^*$  means an increase in black color (decrease in white color) and an increase in  $L^*$  means an increase in white color (decrease in black color).

$a^*$  gives the change in red/green, and a decrease in  $a^*$  means an increase in green color (decrease in red color), and an increase in  $a^*$  means an increase in red color (decrease in green color).

$b^*$  gives the change in blue/yellow, and a decrease in  $b^*$  means an increase in blue color (decrease in yellow color), and an increase in  $b^*$  means an increase in yellow color (decrease in blue color) (Vide WO 96/12846 NOVO).



The Nippon Denshoku's spectrophotometer (SE 2000) was operated in the L\*a\*b\* color space. The light source was D65 standard light. The software used for evaluation was ColorMate Version 4.05. The illumination and light-receiving conditions of this instrument is 0-45° after spectrum method based on JIS Z-8722 and was calibrated using the white and black tiles. Each result was an average of 4 measurements. Fabric rinsed without enzyme and mediator was measured and the coordinates L\*a\*b\* were calculated and entered as a reference. The coordinates of the samples were then for each of L\*, a\*, b\* calculated as the difference of the average of the measurements on each swatch from the reference value.

The present invention is further illustrated in the following examples, which are not in any way intended to limit the scope of the invention as claimed.

### EXAMPLE 1

#### Comparison of anti-back staining effect between Cutinase A and Endolase

An Indigo solution was prepared by washing denim with model washing agent. The compositions of model washing agent are as follows:

Sodium dihydrogen phosphate: 6.2 g/20L

Sodium citrate: 5.8 g/20L

Novasol P: 2.4 g/20L

Carezyme 1000L (available from Novozymes A/S): 2.8 g/20L

The washing conditions were as follows:

Temperature:	55°C
Washing Time:	120 min
Enzyme:	Model washing agent
Enzyme dosage:	1 g/L
Washing liquor:	Deionized water (3°dH) / 20L
Denim:	Kurabo KD511
Bath ratio:	1:20
Washing machine:	Wascator (FOM71MP-Lab.)

Swatches (10 cm x 10 cm) of polyester and polyester/cotton was washed with the indigo solution (pH=6.5) prepared above with Cutinase A and a cellulase (Denimax® 362S), respectively. The conditions were:

Temperature:	55°C
Washing Time:	60 min
Washing liquor:	Indigo solution (pH=6.5)
Enzymes:	Cutinase A and Endolase (Novozym® 613, 3090 ECU/g)
Enzyme dosage:	0, 1, 3, 5, 10 mg enzyme protein/L

Swatch: Polyester, Polyester/Cotton  
Swatch size: 10 cm X 10 cm  
Bath ratio: (Polyester X 2, Polyester/Cotton X 2)/L  
T-O-M: 120 rpm

## Results:

**Table 1. Comparison of anti-back staining effect  
between Cutinase A and Endolase (L\* value)**

Enzyme	Textile	0 mg/L <sup>*1</sup>	1 mg/L <sup>*1</sup>	3 mg/L <sup>*1</sup>	5 mg/L <sup>*1</sup>	10 mg/L <sup>*1</sup>
Cutinase A	Polyester	68.4 +/- 0.8	71.6 +/- 0.2	75.7 +/- 0.2	79.6 +/- 0.4	84.0 +/- 0.2
	Polyester/Cotton	71.8 +/- 0.1	71.8 +/- 0.1	73.9 +/- 0.1	74.5 +/- 0.1	76.9 +/- 0.2
Endolase	Polyester	68.4 +/- 0.8	69.6 +/- 0.3	68.8 +/- 0.5	69.3 +/- 0.4	68.6 +/- 0.4
	Polyester/Cotton	71.8 +/- 0.1	72.4 +/- 0.2	72.7 +/- 0.2	72.1 +/- 0.1	72.7 +/- 0.4

<sup>\*1</sup> Enzyme protein base

The above results show a significant anti-back staining effect on polyester and polyester/cotton of the cutinase compared with the cellulase. The cellulase did not show any anti-back staining effect on the fabric swatches.

## EXAMPLE 2

### Anti-back staining effect of Cutinase A and B and Lipolase.

An Indigo solution was prepared by washing denim with Denimax® 362S in deionised water. The conditions were as follows:

Temperature: 55°C  
Washing Time: 120 min  
Enzyme: Denimax® 362S  
Enzyme dosage: 1 g/L  
Washing liquor: Deionized water (3°dH) / 20L  
Denim: Kurabo KD511  
Bath ratio: 1:20  
Washing machine: Wasicator (FOM71MP-Lab)

Swatches (10 cm x 10 cm) of polyester and polyester/cotton was washed with the indigo solution (pH=6.5) prepared above with the cutinases and Lipolase® 100L (available from Novozymes A/S), respectively. The conditions were:

Temperature: 55°C  
Washing Time: 60 min

Washing liquor: Indigo solution (pH=6.5)  
 Enzymes: Cutinase A and B and Lipolase® 100L, type EX  
 Enzyme dosage: 0, 10, 30, 50 mg enzyme protein/L (Table 2) and 0, 1, 3, 5 mg enzyme protein/L (Table 3)  
 Swatch: Polyester and Polyester/Cotton  
 Swatch size: 10 cm X 10 cm  
 Bath ratio: (Polyester X 2, Polyester/Cotton X 2) /L  
 T-O-M: 120 rpm

## Results:

Table 2. Anti-back staining effect of enzymes on polyester and polyester/cotton (L\* value)

Textile	Enzyme	0 mg/L* <sup>1</sup>	10 mg/L* <sup>1</sup>	30 mg/L* <sup>1</sup>	50 mg/L* <sup>1</sup>
Polyester	Cutinase A	65.4 +/- 0.3	84.6 +/- 0.1	89.0 +/- 0.0	89.7 +/- 0.1
	Cutinase B	65.7 +/- 0.2	88.3 +/- 0.1	90.3 +/- 0.2	90.8 +/- 0.2
	Lipolase	65.1 +/- 0.2	66.0 +/- 0.4	68.1 +/- 0.2	69.0 +/- 0.4
Polyester/Cotton	Cutinase A	67.9 +/- 0.3	76.3 +/- 0.2	83.3 +/- 0.0	84.6 +/- 0.1
	Cutinase B	68.6 +/- 0.1	82.3 +/- 0.1	86.7 +/- 0.1	86.9 +/- 0.1
	Lipolase	68.1 +/- 0.3	69.2 +/- 0.1	71.8 +/- 0.1	73.6 +/- 0.2

\*<sup>1</sup> Enzyme protein base

Table 3. Anti-back staining effect of cutinase with low enzyme dosage (L\* value)

Textile	Enzyme	0 mg/L* <sup>2</sup>	1 mg/L* <sup>2</sup>	3 mg/L* <sup>2</sup>	5 mg/L* <sup>2</sup>
Polyester	Cutinase A	63.7 +/- 0.1	68.6 +/- 0.5	75.9 +/- 0.6	81.2 +/- 0.4
	Cutinase B	63.9 +/- 0.2	71.0 +/- 0.1	76.3 +/- 0.3	79.3 +/- 0.6
Polyester/Cotton	Cutinase A	64.2 +/- 0.1	65.5 +/- 0.2	67.5 +/- 0.4	71.0 +/- 0.1
	Cutinase B	64.5 +/- 0.2	66.3 +/- 0.2	69.7 +/- 0.4	72.2 +/- 0.3

The above results show an anti-back staining effect on polyester and polyester/cotton of the cutinases and the Lipolase.

## EXAMPLE 3

### Anti-back staining effect of cutinase and lipases at acid pH condition

An Indigo solution was prepared by washing denim with Cellusoft® L in deionised water. The conditions were as follows:

Temperature: 55°C

Washing Time: 120 min  
 Enzyme: Cellusoft® L  
 Enzyme dosage: 1 g/L  
 Buffer: 1 M Acetate buffer (pH=4.8) / 100 ml/20 L  
 Washing liquor: Deionized water (3°dH) / 20 L  
 Denim: Kurabo KD511  
 Bath ratio: 1:20  
 Washing machine: Wasicator (FOM71MP-Lab.)

Swatches (10 cm x 10 cm) of polyester and polyester/cotton was washed with the indigo solution (pH=5) prepared above with Cutinase A and B, Lipolase® and Lipolase™ Ultra, respectively. The conditions were:

Temperature: 55°C  
 Washing Time: 60 min  
 Washing liquor: Indigo solution (pH=5)  
 Enzymes: Cutinase A and B, Lipolase® and Lipolase™ Ultra  
 Enzyme dosage: 0, 10, 30, 50 and 100 mg enzyme protein/L (Table 4) and 0, 10 and 30 mg enzyme protein/L (Table 5)  
 Swatch: Polyester, Polyester/Cotton  
 Swatch size: 10 cm X 10 cm  
 Bath ratio: (Polyester X 2, Polyester/Cotton X 2)/L  
 T-O-M: 120 rpm

Results:

Table 4. Anti-back staining effect of Cutinase A and B (L\* value)

Enzyme	Textile	0 mg/L <sup>*1</sup>	10 mg/L <sup>*1</sup>	30 mg/L <sup>*1</sup>	50 mg/L <sup>*1</sup>	100 mg/L <sup>*1</sup>
Cutinase A	Polyester	68.8+/-0.5	74.7+/-1.0	79.6+/-0.2	79.9+/-0.1	82.2+/-1.6
	Polyester/Cotton	65.3+/-0.9	66.8+/-0.2	68.1+/-0.6	67.5+/-1.0	69.9+/-0.6
Cutinase B	Polyester	66.7+/-0.3	80.3+/-0.2	82.4+/-0.6	82.7+/-0.2	81.7+/-0.6
	Polyester/Cotton	67.4+/-0.9	69.2+/-0.4	69.2+/-1.0	70.6+/-0.1	70.8+/-0.5

<sup>\*1</sup> Enzyme protein

Results:

Table 5. Anti-back staining effect of Cutinase A and B,  
Lipolase® and Lipolase™ Ultra (L\* value)

Textile	Enzymes	0 mg/L <sup>*1</sup>	10 mg/L <sup>*1</sup>	30 mg/L <sup>*1</sup>
Polyester	Cutinase A	68.5+/-0.4	77.9+/-0.5	81.9+/-0.5
	Cutinase B	Ditto	79.6+/-0.9	81.5+/-0.3
	Lipolase®	Ditto	80.0+/-0.8	81.5+/-0.9
	Lipolase™ Ultra	Ditto	75.5+/-0.4	75.6+/-0.4
Polyester/Cotton	Cutinase A	62.0+/-0.8	62.6+/-0.6	63.7+/-0.6
	Cutinase B	Ditto	63.8+/-0.5	64.3+/-0.7
	Lipolase®	Ditto	63.5+/-0.5	67.3+/-0.2
	Lipolase™ Ultra	Ditto	63.2+/-0.5	65.2+/-0.6

The above results show an anti-back staining effect of the cutinases and the lipases on polyester and polyester/cotton at acidic pH.